Lipase Activity in Blood from the Hepatic and Peripheral Vascular Beds Following Heparin.* (28598)

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A lipolytic and lipemia clearing enzyme appears in plasma soon after intravenous heparin injection(1). An enzyme with similar activities can be demonstrated in adipose tissue and cardiac muscle by various *in vitro* procedures. The active lipase obtained and assayed by different methods has been variously called lipoprotein lipase, postheparin plasma lipase and clearing factor lipase. The latter term, clearing factor lipase (CFL) was selected for the present report on the basis of the experimental model employed.

Robinson and French(2) suggested that CFL occurs normally on the capillary surface and that the rôle of heparin is one of displacing the enzyme into the circulating blood. Robinson and Harris(3) further suggested that the vascular wall component responsible for production of CFL may be distributed generally in the walls of blood vessels and not localized in any particular organ. The immediate appearance of CFL in the vascular beds of the rabbit hindlimb(3) and human forearm(4,5) in response to heparin are consistent with this hypothesis. However, attempts to demonstrate CFL in the liver have produced conflicting results. With one possible exception(6), early studies, all utilizing *in vitro* methods, failed to demonstrate the presence of CFL in the liver(7,8,9). Barclay *et al.*(10), reported extraction of a lipolytic enzyme with properties similar to CFL from the livers of normal rats, and the livers and tumors of rats with Walker carcinosarcoma 256.

Using an *in vivo* experimental model fashioned after that of Robinson and Harris(3), the immediate release of CFL into peripheral vascular beds in response to heparin has been confirmed in man and dog. In addition, heparin-induced release of CFL into the hepatic vascular bed of the dog has been demonstrated.

Methods. Lipolytic activity and clearing of a triglyceride substrate were assayed in plasma or serum from blood withdrawn during the initial 20 seconds following injection of sodium heparin (Abbott) into the circulation of the human forearm, the hindlimb of the dog and liver of the dog. These plasma samples are referred to as 20 second postheparin samples, which means that sampling was stopped 20 seconds after beginning injection. Lipolytic and clearing activity in preheparin samples and samples withdrawn at 2 and 5 minutes postheparin were similarly assayed. All samples were centrifuged in the cold at 4000 *g* for 15 minutes and maintained at 0°C until assayed on the same day.

In the human forearm experiments, 12 patients undergoing routine arterial puncture for cardio-pulmonary study were injected rapidly (2-3 seconds) with 10 mg of heparin into the brachial artery. Fifteen ml blood samples were withdrawn from the ipsilateral antecubital vein.

Twenty-one experiments utilizing the circulation of the dog hindlimb were similarly performed with injection of 10 mg of heparin into the femoral artery and sampling from the ipsilateral femoral vein.

Ten of the above dogs subsequently (2-3 weeks) had radio-opaque catheters introduced into the inferior vena cava via the femoral vein and placed just cephalad to the entry of the hepatic veins. A control sample was withdrawn through the catheter and 10 mg of heparin was rapidly injected (2-3 seconds) into the portal vein which had been exposed previously by abdominal laparotomy. Samples were removed from the vena cava by way of the catheter. Thirty ml blood samples were withdrawn in all experiments utilizing the dog hindlimb and the dog liver. No evidence of shock as a result of blood loss was observed.

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TABLE I. Summary of Postheparin Lipolytic Activity Developed in Vascular Beds of Human Forearm, Dog Hindlimb and Dog Liver.*

Type of experiment	Preheparin, mEq/l FFA†	Postheparin plasma, mEq/l FFA†		
		20 sec	2 min	5 min
Forearm circulation, 12 humans	.19 ± .18 (0–.5)	1.41 ± .95 (.42–3.82)	7.12 ± 1.49 (3.96–8.62)	9.13 ± 2.27 (5.04–12.07)
Hindlimb circulation, 21 dogs	.12 ± .16 (0–.43)	1.38 ± .70 (.53–2.85)	7.25 ± 2.42 (2.36–10.69)	8.97 ± 2.77 (3.47–12.84)
Hepatic circulation, 10 dogs	.14 ± .13 (0–.62)	3.38 ± .93 (2.84–4.81)	8.37 ± 1.87 (4.03–11.32)	10.19 ± 2.26 (4.32–12.90)

* Values are given as mean ± 1 standard deviation with range below in parentheses.
† Incubation for 3 hr at 37°C.

Sodium pentobarbital (Nembutal) anesthesia (35 mg/kg) was used in all dog experiments.

The possibility of CFL release at sites removed from the vascular bed under study, due to recirculation of heparin, was eliminated. In the experimental model utilizing the dog hindlimb, this was done by injecting indocyanine green (cardio-green, Hynson, Wescott and Dunning) into the contralateral femoral vein simultaneously with heparin injection. No dye appeared in the 20 second postheparin sample. In the experiments utilizing the liver, this was done by injecting the dye into the carotid artery and withdrawing samples at 10, 20, 30 and 60 seconds from a catheter placed in the inferior vena cava at the level of the hepatic veins. No dye appeared in samples taken within the initial 20 seconds after beginning injection as determined spectrophotometrically. Dye concentrations were determined in a Model B Beckman spectrophotometer at 800 mμ.

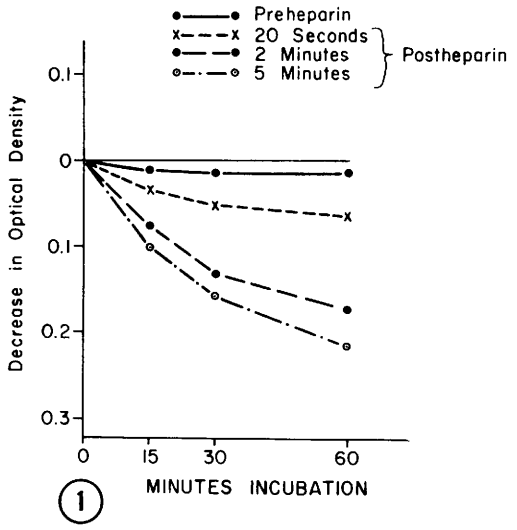
Lipolytic activity was determined by measuring the amount of free fatty acid (FFA) released from a triglyceride substrate (Ediol, Schenley) during 3 hours incubation at 37°C. Each ml of the incubation mixture contained 0.7 mg coconut oil, 0.2 ml of test plasma and 15.0 mg human serum albumin adjusted to a pH of 8.0 with 0.066 M phosphate buffer. Initial and terminal aliquots were withdrawn from the incubation mixture for extraction and titration of long chain free fatty acids by the method of Dole as modified by Trout (11). The results are expressed as milliequivalents of free fatty acid per liter.

Clearing activity was determined by measuring the decrease in optical density of a triglyceride substrate (Ediol) in a model B. Beckman Spectrophotometer at 660 mμ. Each ml of the incubation mixture contained 0.125 mg coconut oil and 0.05 ml of test plasma adjusted to a pH of 8.0 with 0.066 M phosphate buffer.

Results. Development of lipolytic activity, human forearm. Development of clearing factor lipase (CFL) in the vascular bed of the human forearm, as evidenced by the lipolytic activity of postheparin plasma, was studied in 12 male patients. In Table I it can be seen that 20 seconds postheparin plasma in this group brought about the release of an average of 1.41 meq per l FFA, which is significantly greater than the preheparin average ($P < .001$). The 2 and 5 minute postheparin plasmas caused the release of an average of 7.12 meq per l and 9.13 meq per l FFA respectively.

Dog hindlimb and liver. Table I provides a comparison of the amounts of CFL released by the vascular beds of the dog hindlimb and liver within 20 seconds after heparin injection. It was found that the 20 second postheparin plasma from the vascular bed of the hindlimb effected the release of an average of 1.38 meq per l FFA. Twenty second postheparin plasma from the hepatic vascular bed of 10 dogs yielded an average release of 3.38 meq per l FFA. This amount is significantly greater than the average for the hindlimb values cited above ($P < .001$). In both hindlimb and hepatic series, the mean 20 second postheparin plasma lipolytic activities were significantly greater than corresponding preheparin averages ($P < .001$).

In a group of 10 dogs, the heprain-induced



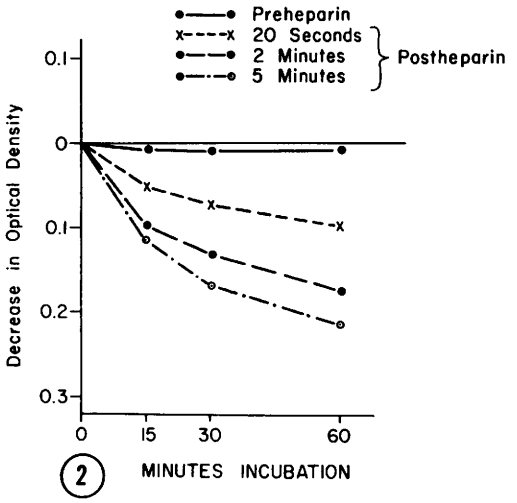


FIG. 1. Mean clearing activity developed in plasma from vascular bed of hindlimb in 14 dogs at 20 sec and at 2 and 5 min postheparin.

FIG. 2. Mean clearing activity developed in plasma from vascular bed of liver in 9 dogs at 20 sec and at 2 and 5 min postheparin.

release of CFL was studied first in the vascular bed of the hindlimb and subsequently in that of the liver. The results obtained from these paired experiments were statistically similar to those derived from the pooled data cited above (Table I). Twenty second postheparin plasmas from all hindlimb and hepatic vascular beds demonstrated significant lipolytic activity. However, the lipolytic activity which appeared from the hepatic circulation within 20 seconds after injection was invariably in excess of the amount which appeared from the vascular bed of the hindlimb in the same dog. At both 2 and 5 minutes postheparin, there was no significant difference in the lipolytic activity from hindlimb as compared to hepatic vascular bed.

Optical density studies. Dog hindlimb and liver. Development of CFL activity in the vascular bed of the dog hindlimb, as evidenced by the ability of postheparin plasma to clear a triglyceride substrate, was studied in 14 animals. In Fig. 1 it can be seen that the 20 second postheparin plasma from the vascular bed of the dog hindlimb effected an average clearing of the triglyceride substrate on 60 minutes incubation, which was significantly greater than that effected by the preheparin controls ($P < .001$). Plasma obtained from blood samples taken at 2 minutes postheparin were more active while plasma obtained from samples taken 5 minutes postheparin invariably exhibited even greater clearing activity.

Development of CFL in the vascular bed of the liver, as evidenced by the clearing activity of postheparin plasma, was studied in 9 animals. In Fig. 2 it can be seen that 20 second postheparin plasma from the hepatic circulation caused clearing of the triglyceride substrate which was more pronounced than that caused by 20 second postheparin plasma from the hindlimb ($P < .05$). At both 2 and 5 minutes postheparin, there was no significant difference in the clearing activity from hindlimb as compared to hepatic vascular bed.

Discussion. The *in vivo* experiments reported here demonstrate that clearing factor lipase (CFL) is released into the blood stream during a single passage of heparin through the vascular beds of the human forearm, dog hindlimb and dog liver. These findings support the hypothesis that CFL is released rapidly from the endothelium or some other component of the vascular wall(1,2,3). Because of the liver's extensive capillary bed, the high titer of CFL released by the liver in response to heparin is consistent with this hypothesis. The demonstration of heparin-induced CFL released by the liver indicates

that this enzyme may have a specific rôle in fatty acid transport.

Summary. Clearing factor lipase, an enzyme with the ability to clear a lipemic substrate and to cause hydrolysis of triglyceride, is released during a single passage of heparin through the vascular beds of the dog liver, dog hindlimb and human forearm.

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Effect of Reserpine and Guanethedine on Excretion of 3-Methoxy-4-Hydroxymandelic Acid in Man.* (28599)

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Rapid depletion of catecholamine tissue stores follows parenteral administration of large doses of reserpine and guanethedine in animals. The mechanism by which this depletion occurs is unsettled. Depletion of catecholamine stores in man following doses employed therapeutically has not been proven. 3-methoxy-4-hydroxymandelic acid (VMA) has been shown to be a specific and major metabolic product of norepinephrine (NE) and epinephrine (E). VMA, which can be precisely measured, appears in the urine in quantities 50 to 100 times greater than NE plus E.

Large intravenous doses of reserpine and guanethedine were therefore administered to human subjects and urinary VMA was measured in order to 1) seek evidence for or against release of catecholamines from body depots and subsequent increased excretion; and 2) to compare the effects of the two drugs.

Methods. Thirty-two subjects were selected, of which 16 were normal volunteers and 16 had uncomplicated essential hypertension established by appropriate studies. One-half were women and half were men. Since no difference in VMA excretion in response to reserpine and guanethedine was found between normotensive and hypertensive subjects, the data from these groups were combined, and 16 whose urine was collected at 8 hour intervals are presented here. No medication had been taken for at least 6 weeks prior to study. During the investigative period, all subjects were placed on a diet containing no fruit, coffee, tea, vanilla or chocolate. The experimental design is shown in Table I. All drugs and placebos were infused during a 15 minute period between 8 and 9 AM. During the infusion and for 45 minutes subsequently, blood pressure was taken every 2 minutes, followed by measurements every 4 hours for the next 23 hours. The cuff method was used. After measurement of urinary pH and volume, creatinine was estimated by means of automatic chemical analyses(1). Aliquots for VMA determination were preserved with a crystal of thymol at −10°C. VMA was determined quantitatively

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